ZOOLOGICAL SCIENCE 6: 789–796 (1989)



# Cytological Differences in Early Germ Cells of the Three Genera of Grey Mullets, *Mugil, Liza* and *Chelon* (Teleostei: Mugilidae)

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**ABSTRACT**—A comparative study of the fine structure of early germ cells, primordial germ cells, oogonia and spermatogonia, of the three genera *Mugil, Liza* and *Chelon* of grey mullets has allowed the former to be separated from the other two genera.

## INTRODUCTION

Among the most interesting works on the taxonomy of mugilids, we noticed those of Schultz [1], FAO [2], Thomson [3, 4] and Trewavas [5]. Several keys of determination for different species of grey mullets in the Mediterranean have been published by De Angelis [6], Tortonese [7], Ben Tuvia [8, 9], Farrugio [10], and Cambrony [11]. At present, six Mediterranean species were known:

*Mugil cephalus cephalus* (Linaeus, 1758)–Clofnam 181.1.

*Chelon labrosus* (Risso, 1826)–Clofnam 101.21.

*Liza (Liza) ramada* (Risso, 1826)–Clofnam 181.3.1

*Liza (Liza) aurata* (Risso, 1810)–Clofnam 181.3.2.

*Liza (Protomugil) saliens* (Risso, 1810)–Clofnam 181.3.4.

*Oedalechilus labeo* (Cuvier, 1829)–Clofnam 101.4.1.

Specific differences in grey mullets concern morphological characteristics [4, 10, 12], morphometric [13] and anatomical evidences [4, 10, 14]. Moreover, research has been carried out in kariology [15], biology [16–18] and parasitology [19, 20]. The most interesting findings concern the genetic results in the field of blood proteins [21–23], serous proteins [24], proteins of the cristallin lens [25] and enzymatic polymorphism [23, 26]. From all these results, differences have been established among the three genera *Mugil, Liza* and *Chelon* [4, 13]. No cytological information has been made in literature. A comparative study of gonadogenesis and gametogenesis in Mediterranean grey mullets [27] has allowed us to obtain ultrastructural knowledge on germ cells ranging from primordial germ cells [27, 28] to male [29] and female [30] gametes.

The present study therefore deals with the ultrastructure of the early germ cells in *Liza aurata, Mugil cephalus* and *Chelon labrosus* which are the most common species among the six known in Roussillon (Golf of Lion).

Accepted October 24, 1988
Received July 13, 1988

## MATERIALS AND METHODS

Juvenile grey mullets were caught by electric fishing in the brackish waters of Leucate, a Mediterranean lagoon and adult grey mullets by trawl in the Mediterranean Sea. Gonads were fixed for electron microscopy by the aid of conventional procedure previously reported [31].

## RESULTS

Eary germ cells, primordial germ cells (PGCs), oogonia and spermatogonia were examined in

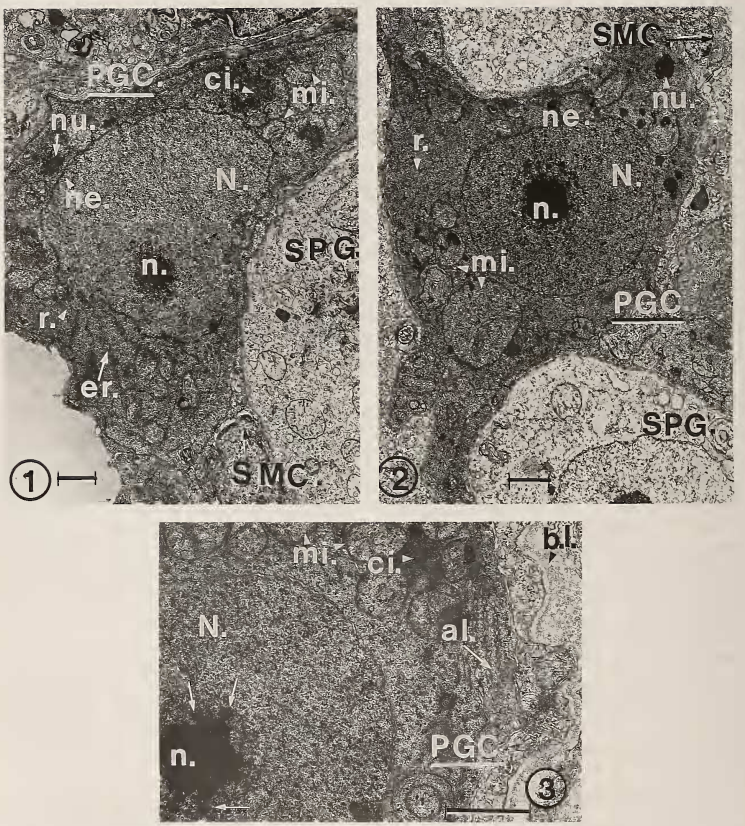



FIG. 1. PGC in *Liza aurata*.
FIG. 2. PGC in *Mugil cephalus*.
FIG. 3. A part of a PGC in *M. cephalus*: annulated lamellae and granules (arrows) budded by fibrillar center of nucleolus.

| | | | |
|---|---|---|---|
| al. | :annulated lamellae | nu. | : "nuage" |
| bl. | : basal lamina | OOG. | : Oogonium |
| ch. | : chromatin | PFC. | : Prefollicle cell |
| ci. | : "ciment" | PGC. | :Primordial germ cell |
| er. | : endoplasmic reticulum | r. | :ribosome |
| mi. | : mitochondria | SC. | : Sertoli cell |
| N. | : Nucleus | SMC. | : Somatic cell |
| n. | : nucleolus | SPG. | : Spermatogonium |
| ne. | : nuclear envelope | | |

The bar represents one micron in all micrographs.

three species of grey mullets, but results are given only for *Liza aurata* and *Mugil cephalus* because no significant differences were detected between *L. aurata* and *C. labrosus*. In *L. aurata,* 125 fish were studied from which micrographs of 71 PGCs, 64 oogonia and 90 spermatogonia were examined and in 82 samples of *M. cephalus,* micrographs of 53 PGCs, 52 oogonia and 52 spermatogonia were observed. These three cell types show the same ultrastructural features at every step of gametogenesis. PGCs developing to oogonia and spermatogonia were previously described [28] and are not noticed in this paper.

*Primordial germ cells (PGCs)*

PGCs (Figs. 1, 2) are oval-shaped undifferentiated cells [27], characterized by irregular outlines (which probably indicate ameboid movements during migration to the gonadal anlage), a high nucleus to cell ratio about diameter (N/C=0.39–0.40), a heavy electron density (owing to a finely granular chromatin and many free ribosomes) and a small number of membrane organelles (endoplasmic reticulum and Golgi complexes). Mitochondria, few in number, are often found with "ciment" (cement) which is a dense fibrillar material (called "nuage" = halo when it is independent of mitochondria). The ciment [32, 33] migrates from the nucleus, is characteristic of germ cells and can also be called "germinal dense bodies" [34]. PGCs are, surrounded by somatic cells, characterized by a high nucleo/cell, a dense chromatin lying along the inner membrane of the nuclear envelope and few membrane organelles. These cells are generally devoid of nucleoli.

PGCs show these typical characteristics in two species. The differences identified are related both to the sizes of the cell and mitochondria and to the structure of mitochondria. In *L. aurata* (Fig. 1), PGCs are 12.1 $\mu$m ($\pm$0.65) in length and 9.0 $\mu$m ($\pm$0.54) in width, and mitochondria are 0.66 $\mu$m in diameter and display short cristae. In *M. cephalus* (Fig. 2), PGCs are smaller (9.58 $\mu$m $\pm$0.63 in length ; 6.03 $\mu$m $\pm$0.63 in width), mitochondria bigger (0.7–1.2 $\mu$m) and mitochondrial cristae narrower than those of *L. aurata*. Moreover, the nuclear envelope of PGCs in *M. cephalus* is more regular (Fig. 2) than that of *L. aurata* (Fig. 1). At last, in *M. cephalus,* rather voluminous granules in cortex of the nucleolus, budded by fibrillar center, and annulated lamellae in the cytoplasm can be observed (Fig. 3).

*Oogonia*

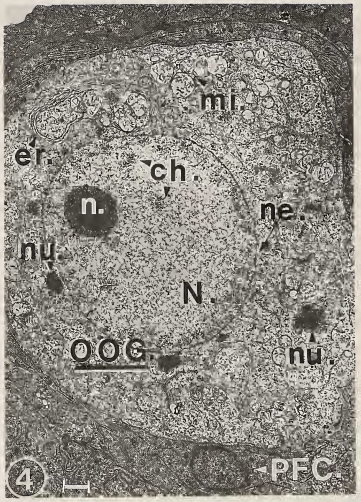
Oogonia (Figs. 4, 5) are oval or round cells characterized by regular outlines, a high nucleus to cell ratio (0.4) and a low electron density owing to a decrease in the number of ribosomes and a rather dispersed chromatin. "Ciment" and "nuage" are still recognized and the spherical nucleus is quite centrally located. There are more numerous membrane organelles than in PGCs. Prefollicle cells surrounding oogonia have already been noticed [35].

These general features of oogonia are seen both in *L. aurata* (Fig. 4) and in *M. cephalus* (Fig. 5) but, in the former species, oogonia are 14.3 $\mu$m ($\pm$1.05) in length and 11.34 $\mu$m ($\pm$1.62) in width, whereas they are 11.9 $\mu$m ($\pm$0.93) long and 9.36 $\mu$m ($\pm$0.75) wide in the latter species. Membrane organelles are more numerous in *L. aurata* than in *M. cephalus*. Moreover, the mitochondria in *L. aurata* are smaller (0.66 $\mu$m in diameter) with shorter cristae than those in *M. cephalus* (0.8–1.5 $\mu$m in diameter). Annulated lamellae are discernible in the oogonia of *M. cephalus*.

*Spermatogonia*

Spermatogonia (Figs. 6, 7) are oval-shaped cells characterized by quite regular outlines, and, like oogonia, have a high nucleus to cell ratio (0.39) and a low electron density. The oval-shaped nucleus is eccentric and the chromatin appears to be rather granular and dispersed, but small dense clumps with the nucleolus are found and packed along the inner nuclear membrane. Cytoplasmic characteristics are quite similar to those noted in oogonia. Sertoli cells surrounding spermatogonia are noticed as was accounted previously [27].

Spermatogonia show the same general characteristics in both species of grey mullets, but in *L. aurata* spermatogonia are 14.2 $\mu$m ($\pm$0.99) in length and 10.5 $\mu$m ($\pm$0.98) in width, and in *M. cephalus* they are 12.42 $\mu$m ($\pm$1.12) and 8.66 $\mu$m ($\pm$1.02) respectively. Differences in the membrane organelles of the two species, similar to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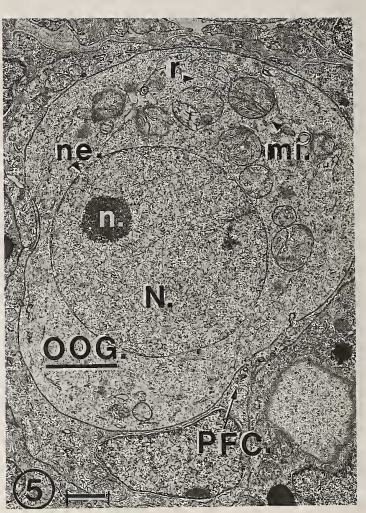
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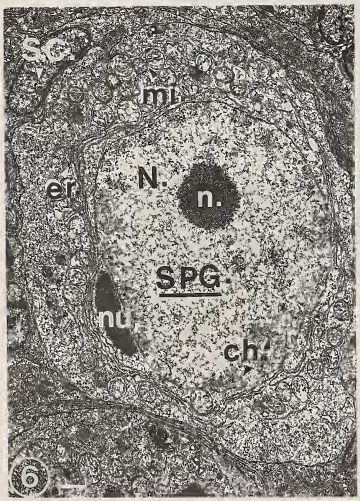
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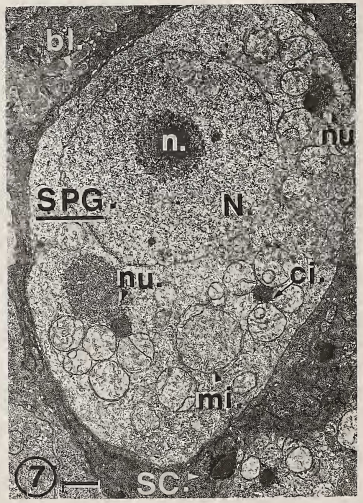
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TABLE 1. Comparative study of early germ cells in *Liza aurata* and *Mugil cephalus*

| | | | Likenesses | Differences | | t | k |
|---|---|---|---|---|---|---|---|
| | | | | *Liza aurata* | *Mugil cephalus* | | |
| PGC | Morphology | | -oval-shaped<br>-heavy electron density<br>-irregular outlines | -long: 12.1 $\mu$m±0.65<br>-wide: 9.0 $\mu$m±0.54 | -long: 9.58 $\mu$m±0.63<br>-wide: 6.03 $\mu$m±0.63 | 26.7<br>25.2 | |
| | Cytology | Nucleus | -voluminous nucleus<br>-N/C=0.39–0.40<br>-finely granular chromatin | -irregular envelope | -rather regular nuclear envelope<br>-fibrillar center budding | | 122 |
| | | Cytoplasm | -numerous ribosomes<br>-scarce membrane organelles<br>-"nuage" and "ciment" | -mitochondria: diameter= 0.66 $\mu$m | -mitochondria: diameter= 0.7–1.2 $\mu$m narrow cristae<br>-annulated lamellae | | |
| OOGONIA | Morphology | | -oval or round-shaped<br>-low electron density<br>-regular outlines | -long: 14.3 $\mu$m±1.05<br>-wide: 11.34 $\mu$m±1.62 | -long: 11.9 $\mu$m±0.93<br>-wide: 9.36 $\mu$m±0.75 | 13.1<br>8.2 | |
| | Cytology | Nucleus | -round, central and voluminous nucleus N/C=0.40<br>-granules of chromatin dispersed<br>-regular outlines | | | | 114 |
| | | Cytoplasm | -ribosomes dispersed<br>-"nuage" and "ciment" | -membrane organelles rather numerous<br>-mitochondria: diameter= 0.66 $\mu$m | -membrane organelles less numerous<br>-annulated lamellae<br>-mitochondria: diameter= 0.8–1.5 $\mu$m narrow cristae | | |
| SPERMATOGONIA | Morphology | | -oval shaped<br>-low electron density<br>-rather regular outlines | -ling: 14.2 $\mu$m±0.99<br>-wide: 10.5 $\mu$m±0.98 | -long: 12.42 $\mu$m±1.12<br>-wide: 8.66 $\mu$m±1.02 | 9.4<br>10.7 | |
| | Cytology | Nucleus | -eccentric and voluminous nucleus N/C=0.4<br>-granules of chromatin dispersed<br>-"nuage" and "ciment" | | | | 140 |
| | | Cytoplasm | -ribosomes dispersed<br>-"nuage" and "ciment" | -TER rather frequent<br>-mitochondria (id. oogonia) | -GER infrequent<br>-annulated lamellae<br>-mitochondria (id. oogonia) | | |

t=student's parameter, k=degree of freedom

FIG. 4. Oogonium in *L. aurata*.
FIG. 5. Oogonium in *M. cephalus*.
FIG. 6. Spermatogonium in *L. aurata*.
FIG. 7. Spermatogonium in *M. cephalus*.

The bar represents one micron in all micrographs.

those noted for oogonia, are discriminated in spermatogonia especially for the endoplasmic reticulum.

All these findings related to the three types of early germ cells in two species studied are summarized in the Table 1.

## DISCUSSION

In the present work, ultrastructural features which allow the identification of PGCs, oogonia and spermatogonia were found to be similar in the three species of grey mullets. These three cell types show the same ultrastructural characteristics at every step of gametogenesis. As cell differentiation proceeds, changes in cell organells are detected from primary cytes [27]. Therefore differences demonstrated between *M. cephalus* on the one hand and *L. aurata* and *C. Labrosus* on the other hand, are rather significant since they do not reflect physiological differences between cells. These differences are recognized in morphological (different sizes of cells, nuclei and mitochondria, shape of nuclear envelope), structural (granular cortex of the nucleolus, mitochondrial cristae) and quantitative (varying numbers of membrane organelles, especially for the endoplasmic reticulum, and presence or absence of annulated lamellae) evidences. Therefore, this study allows the genus *Mugil* to be separated from the other two genera *Liza* and *Chelon,* but there are no cytological criteria available to distinguish *Liza* from *Chelon*. Our results are in accordance with other works which have shown that the genus *Mugil* is quite different from the other two [13, 15, 36]. Especially, biochemical data showed not only this clear separation of the genus *Mugil* [22, 26], but also differences between *Liza* and *Chelon* [21] and the homogeneity of the genus *Liza* [26].

Cytological criteria to make comparisons between different genera or species are generally related to structural characteristics of differentiated cells such as spermatozoa [37, 38] or oocytes [39, 40]. In our previous examinations, significant differences were noted between the spermatozoa of *M. cephalus* and *L. aurata* [27] and between the oocytes of *L. aurata* and *C. labrosus* [30]. As for early germ cells, intergeneric or interspecific differences have rarely been described. However, Hubert [41] showed differences in the features of the nucleoli in PGCs of species belonging to the genus *Lacerta* and Fujimoto *et al.* [42] distinguished the number of ribosomes of PGCs of man from those of rodents. Moreover, we recognized differences (size of cells and strucutre of mitochondria) in PGCs of teleosts *Serranus cabrilla* and *Serranus hepatus* [27]. To the best of our knowledge, these differences in early germ cells by means to distinguish between certain genera are shown in fish for the first time.

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